5. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

5.1. REPRODUCTIVE EFFECTS

Several reproductive toxicity studies for 1,3-butadiene have been undertaken, starting with a study in rats, guinea pigs, rabbits, and dogs by Carpenter et al., 1944. Two studies by Owen and coworkers were done in rats (Owen et al., 1987; Owen and Glaister, 1990). NTP conducted two chronic reproductive toxicity studies in mice (NTP, 1984; 1993). Hackett and co-workers undertook an acute "sperm head morphology" study in B6C3F1 mice (Hackett et al., 1988a) and a dominant lethal study in CD-1 male mice (Hackett et al., 1988b). Dominant lethal studies, both acute and subchronic, have also been done in CD-1 male mice (Anderson et al., 1993, 1995) and in (102/E1xC3H/E1)F1 mice (Adler and Anderson, 1994).

5.1.1. Carpenter et al., 1944

Four groups, each consisting of 24 albino rats, 12 guinea pigs, 4 rabbits, and 1 dog, were exposed to 0, 600, 2,300, or 6,700 ppm 1,3-butadiene 7.5 h/day, 6 days/week for 8 months in 546-L chambers. Except for the dogs, which were all female (only one in each group), the animals were divided equally between the two sexes. Body weights were measured weekly; blood was analyzed monthly; and urinalysis, blood chemistry, organ weights (kidney and liver), and gross and histopathologic examinations were performed at termination. Males and females were mated, but the authors did not indicate when this occurred relative to the treatment period. No deaths were noted in the exposed animals. Terminal body weights in rats were reduced to 90.5%, 86.3%, and 81.2% in the 600, 2,300, and 6,700 ppm groups, respectively, relative to control body weights. A similar trend was noted for male guinea pigs, and weights for dogs and rabbits fluctuated. No effects on organ weights that could be attributed to exposure to 1,3-butadiene were observed. There were no abnormal findings for hematology values or blood chemistry. Microscopic lesions were not observed in the testes, ovaries, or other organs examined (heart, kidney, skeletal muscle, pancreas, or spleen) except for the liver, in which mild, cloudy swelling was noted in 68% of the animals exposed to 6,700 ppm.

Carpenter et al. (1944) provided a few results regarding fertility of rats, guinea pigs, and rabbits exposed to 1,3-butadiene. Fertility, defined as the number of litters produced within a given time, was reduced in rats, with 3.3, 2.7, 2.5, and 2.6 litters being produced by animals exposed to 0, 600, 2,300, or 6,700 ppm, respectively. Because the results were not analyzed statistically and other details regarding the duration of the mating periods were not presented, it is not possible to conclude that 1,3-butadiene had an effect on fertility in rats. Furthermore, fertility in rats was not affected by exposure to 1,3-butadiene when litter size (8.4 pups/litter at 600 ppm, 7.9 pups/litter at 2,300 ppm, and 7.8 pups/litter at 6,700 ppm) was used as the measure; the

average litter size of the two higher exposure groups was similar to that of the control group. Two male and two female offspring from rats exposed to each concentration were exposed along with the parents. According to the authors, the F_1 controls and the 660-ppm group produced three times as many pups as did the F_1 groups exposed to 2,300 or 6,700 ppm. Too few animals were used to adequately evaluate the fertility of the exposed offspring. Guinea pigs in each exposure group produced 16, 13, 10, and 13 pups, respectively. Rabbits exposed to 600 or 2,300 ppm produced no pups, whereas the controls produced 24 pups and the 6,700 ppm group produced 27 pups. Considering that the highest concentration had no effect on fertility in rabbits, it is doubtful that the lack of fertility at the lower concentrations was due to exposure to 1,3-butadiene.

5.1.2. Owen et al., 1987; Owen and Glaister, 1990

This 2-year toxicological and carcinogenicity study is the same as the Hazleton Laboratories Europe, Ltd. (HLE, 1981), study discussed previously by EPA (U.S. EPA, 1985). Male and female CD strain (Sprague-Dawley derived) rats (110 of each sex per group) were exposed by inhalation to 1,3-butadiene (99.2% purity) at target concentrations of 0, 1,000, or 8,000 ppm 6 h/day, 5 days/week for 105 (females) or 111 (males) weeks. Ten males and 10 females were killed at 52 weeks. The average weekly concentration of 4-vinyl-1-cyclohexene (a 1,3-butadiene dimer) was 413 ppm (v/v). A comprehensive postmortem examination, including necropsy and histopathologic examination, was conducted of all gross lesions, all tissues from control and high-exposure groups, and selected tissues from low-exposure groups. Nonneoplastic lesions were not induced in reproductive organs in either male or female rats, although benign and malignant mammary tumors, uterine sarcomas, and Leydig cell tumors were observed.

5.1.3. NTP, 1984

The first inhalation toxicological and carcinogenicity study conducted by the National Toxicology Program (NTP, 1984) showed that, in addition to the numerous neoplasms induced by high concentrations of 1,3-butadiene in male and female B6C3F_1 mice, nonneoplastic lesions also were induced in reproductive organs. Male and female mice were exposed to 0, 625, or 1,250 ppm 1,3-butadiene 6 h/day, 5 days/week and then killed after 60 or 61 weeks of exposure. Among female mice, ovarian atrophy was seen in 40/45 (89%) mice exposed to 625 ppm and in 40/48 (83%) mice exposed to 1,250 ppm, compared with an incidence of only 2/49 (4%) in control mice. Involution of the uterus, which was considered a manifestation of ovarian atrophy, was seen in 7/46 (15%) and 14/49 (29%) mice exposed to 625 and 1,250 ppm, respectively, compared with 0/49 control mice. Uterine involution was characterized by fewer and less prominent endometrial glands. A low incidence of mammary gland neoplasms (acinar cell and

adenosquamous carcinomas) was induced by 1,3-butadiene; nonneoplastic mammary lesions were not induced. Testicular atrophy was observed in 19/47 (40%) mice exposed to 625 ppm and in 11/48 (23%) mice exposed to 1,250 ppm compared with 0/50 control mice. Statistical analysis showed that the increased incidences of the lesions in male and female mice were significant ($p<0.05$) for all groups compared with their respective controls.

5.1.4. NTP, 1993

NTP (1993) conducted a second inhalation toxicological and carcinogenicity study in male and female B6C3F$_1$ mice exposed to lower concentrations of 1,3-butadiene. Concentrations were 0, 6.25, 20, 62.5, 200, or 625 ppm 1,3-butadiene for 6 h/day, 5 days/week for 103 weeks, with interim evaluations at 9 and 15 months. Additional male mice were exposed to 200 ppm of 1,3-butadiene for 40 weeks, 312 ppm for 52 weeks, or 625 ppm for 13 or 26 weeks followed by observation for the remainder of the 2 years (stop-exposure protocol). It should be emphasized that this study was designed to study neoplastic and general toxicological, rather than reproductive, endpoints. Further details are presented in Chapter 6.

The effects of 1,3-butadiene on reproductive organs in female mice are presented in Table 5-1. Ovarian atrophy was seen in the 200 ppm and 625 ppm exposure groups sacrificed for the 9-month interim evaluation. The atrophic ovaries were characterized by the absence of oocytes, follicles, and corpora lutea. No occurrences of this lesion were noted in the lower exposure groups. Hyperplasia of the germinal epithelium was observed in one animal exposed to 625 ppm for 9 months. Germinal epithelial hyperplasia was described as prominent down growth of the mesothelial surface into the parenchyma of the ovary, forming tubular and gland like structures. At the 15-month interim evaluation, ovarian atrophy was observed in mice exposed to 20 ppm or higher; the incidence at 62.5 ppm or higher was significant compared with concurrent controls. Hyperplasia of the germinal epithelium was seen at 200 and 625 ppm at nonsignificant incidences. Angiectasis (dilation of blood vessels) was seen in one mouse in the control group, one exposed to 6.25 ppm, and two exposed to 200 ppm. The ovary, which was evaluated at 15 months in only two female mice exposed to 625 ppm, was atrophic in both. Among female mice exposed to 1,3-butadiene for 2 years, ovarian atrophy was observed in all exposure groups at incidences that were significantly elevated compared with controls. Therefore, using ovarian atrophy as an endpoint of reproductive toxicity, a no-observed-adverse-effect level (NOAEL) could not be defined in this mouse study. The incidence of angiectasis was significantly elevated only at 62.5 and 200 ppm, and the incidence of germinal epithelial hyperplasia was significantly elevated at 20 to 625 ppm. The occurrence of ovarian atrophy and germinal epithelial hyperplasia showed significant dose-related trends, whereas ovarian

Table 5-1. Reproductive tract lesions in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation

Lesion	Concentration (ppm)					
	0	6.25	20	62.5	200	625
9-Month interim evaluation						
Ovary[a]	10	—	—	10	10	8
Atrophy	0 (0%)	—	—	0 (0%)	9 (90%)[b]	8 (100%)[b]
Germinal epithelial hyperplasia (NOS)	0 (0%)	—	—	0 (0%)	0 (0%)	1 (13%)
Uterus[a]	10	—	—	10	10	8
Atrophy[c]	0 (0%)	—	—	0 (0%)	3 (30%)	6 (75%)
15-Month interim evaluation						
Ovary[a]	10	10	10	10	10	2
Atrophy	0 (0%)	0 (0%)	1 (10%)	9 (90%)[b]	7 (70%)[b]	2 (100%)[d]
Germinal epithelial hyperplasia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (30%)	1 (50%)
Angiectasis	1 (10%)	1 (10%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)
Uterus[a]	10	1	10	10	10	2
Atrophy[c]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
2-Year study[e]						
Ovary[a]	49	49	48	50	50	79
Atrophy	4 (8%) $p<0.001$	19 (39%) $p<0.001$	32 (67%) $p<0.001$	42 (84%) $p<0.001$	43 (86%) $p<0.001$	69 (87%) $p<0.001$
Germinal epithelial hyperplasia	2 (4%) $p<0.001$	3 (6%) $p=0.460$	8 (17%) $p=0.017$	15 (30%) $p<0.001$	15 (30%) $p=0.010$	18 (23%) $p<0.001$
Angiectasis	4 (8%) $p=0.259$	6 (12%) $p=0.366$	3 (6%) $p=0.606$	13 (26%) $p=0.017$	14 (28%) $p=0.021$	17 (22%) $p=0.425$
Uterus[a]	50	49	50	49	50	78
Atrophy[c]	1 (2%)	0 (0%)	1 (2%)	1 (2%)	8 (16%)	41 (53%)

[a]Number of animals for which this site was examined microscopically.
[b]$p<0.01$, pairwise comparison with controls by Fisher's exact test.
[c]Statistical tests were not conducted for these lesions.
[d]$p<0.05$, pairwise comparison with controls by Fisher's exact test.
[e]p values for the statistical analysis (logistic regression test) for the 2-year study are presented; the value for the trend test is in the column for the control group, and the value for pairwise comparisons of individual exposed group with the corresponding control group is in the column for the exposed groups.

Source: NTP, 1993.

angiectasis did not. Although the functional integrity of the female reproductive system was not assessed, it can be assumed that animals without oocytes or follicles would be infertile and would express reduced estrogenic and progestin secretory capacities.

Uterine atrophy was seen at the two highest concentrations at 9 months, but was seen only at the highest concentration at the 15-month evaluation. After 2 years, the incidence of uterine atrophy among mice exposed to 200 and 625 ppm did not increase relative to that observed at 9 months.

Data regarding the effect of 1,3-butadiene on the reproductive organs of male $B6C3F_1$ mice are summarized in Table 5-2. The testes of males exposed to the highest concentration of 1,3-butadiene (625 ppm) were atrophic at the 9- and 15-month interim evaluations and at termination of the 2-year study. Among male mice exposed to 1,3-butadiene in the stop-exposure studies, testicular atrophy was observed in only five mice exposed to 200 ppm (40 weeks), five exposed to 625 ppm (26 weeks), three exposed to 312 ppm (52 weeks), and three exposed to 625 ppm (13 weeks). It is not possible to determine if the lack of a more prominent response in mice exposed to 625 ppm for 26 weeks was due to insufficient time for induction of testicular atrophy or if atrophy had been induced during exposure and the lesion repaired before termination of the stop-exposure study.

5.1.5. Hackett et al., 1988a

This sperm-head morphology study was conducted in $B6C3F_1$ mice at Pacific Northwest Laboratories for NTP as part of a series of studies to investigate the effects of 1,3-butadiene on reproductive function. Twenty male $B6C3F_1$ mice (12 to 13 weeks old) per group were exposed to 1,3-butadiene (99.88% purity; 174 ± 13 ppm mean headspace dimer [4-vinyl-1-cyclohexene] concentration) at concentrations of 0 (filtered air), 200, 1,000, or 5,000 ppm 6 h/day for 5 successive days. Measured concentrations (mean ± standard deviation [SD]) were 199 ± 6.12, 999 ± 22.6, and 4,980 ± 130 ppm. The animals were exposed in a 2.3 m^3 stainless steel chamber with a mixing volume of 1.7 m^3. Positive controls received intraperitoneal injections of 167 mg/kg of ethyl methane sulfonate daily for 5 consecutive days. After exposure, the mice were observed twice daily for mortality, morbidity, and signs of toxicity; body weights were determined weekly. The mice were killed 5 weeks after exposure, weighed, and examined for gross lesions, with particular emphasis on the reproductive tract. Sperm collected from the right epididymis were examined for abnormal heads (blunt hook, banana, amorphous, pinhead, two heads/two tails, short) and other abnormalities (primarily midpiece abnormalities).

Final body weights for the unexposed, treated, and positive control groups were similar, and net body weight gain over the period of the experiment was also similar for all groups. Piloerection and dyspnea were observed within the first 20 to 30 min after exposure in mice

Table 5-2. Reproductive tract lesions in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation

Lesion	Concentration (ppm)					
	0	6.25	20	62.5	200	625
9-Month interim evaluation						
Testes[a]	10	10	10	10	10	10
Absolute weight (g)	0.117 ± 0.002	0.117 ± 0.003	0.114 ± 0.003	0.103 ± 0.004[b]	0.102 ± 0.002[b]	0.059 ± 0.003[b]
Relative weight (mg/g BW)[c]	2.89 ± 0.06	2.92 ± 0.09	2.76 ± 0.09	2.87 ± 0.12	2.54 ± 0.05[b]	1.57 ± 0.03[b]
Atrophy[d]	0 (0%)	C[e]	C	C	0 (0%)	6 (60%)
15-Month interim evaluation						
Testes[a]	10	10	10	10	10	7
Absolute weight (g)	0.116 ± 0.003	0.113 ± 0.003	0.104 ± 0.004	0.112 ± 0.003	0.100 ± 0.003[b]	0.071 ± 0.004[b]
Relative weight (mg/g BW)	2.62 ± 0.07	2.79 ± 0.08	2.48 ± 0.04	2.66 ± 0.07	2.39 ± 0.05[f]	1.80 ± 0.05[b]
Atrophy[d]	0 (0%)	C	0 (0%)	C	0 (0%)	4 (57%)
2-Year study						
Testes[a]	50	50	50	48	49	72
Atrophy[d]	1 (2%)	3 (6%)	4 (8%)	2 (4%)	6 (12%)	53 (74%)

[a]Number of animals for which this site was examined.
[b]$p \leq 0.01$, pairwise comparison with controls by Williams' or Dunnett's test.
[c]BW = body weight.
[d]Statistical tests were not conducted for these lesions.
[e]Testes were not examined microscopically at this concentration.
[f]$p < 0.05$, pairwise comparison with controls by Williams' or Dunnett's test.

Source: NTP, 1993.

receiving 5,000 ppm; no signs of toxicity were noted for the other groups. Exposure-related gross toxicity was not observed in the reproductive tract. The percentages of epididymal sperm with normal morphology were 98.08%, 97.23% ($p<0.05$), and 96.34% ($p<0.05$) at 200, 1,000, and 5,000 ppm, respectively, compared with 98.40% for controls; these values also showed a significant exposure-related trend ($p \leq 0.05$). The percentage of the following abnormalities were significantly elevated compared with controls ($p<0.05$): blunt hooks at 5,000 ppm, bananas at 1,000 and 5,000 ppm, and pinheads at 1,000 ppm. Amorphous, two heads/two tails, and shorts were not significantly elevated at any dose. The predominant types of abnormalities were the banana followed by blunt hook and amorphous. The authors speculated that late spermatogonia or early primary spermatocytes were sensitive to 1,3-butadiene. The authors also stated that examining the sperm at only one time point following termination of exposure precluded a determination of the stage of spermatogenesis affected by the chemical.

5.1.6. Hackett et al., 1988b

This dominant lethal study was conducted using proven breeder male CD-1 mice (20 per group) exposed to 0, 200, 1,000, or 5,000 ppm 1,3-butadiene 6 h/day for 5 successive days. Measured concentrations (mean ± SD) were 200 ± 5.73, 1,010 ± 13.9, and 5,000 ± 85.4 ppm, respectively. The purity of the 1,3-butadiene was 99.88%, and the headspace dimer concentration was 215 ± 49 ppm. For mating, one exposed or control male mouse was placed with two unexposed female mice for 1 week for 8 successive weeks; the two females were replaced each week. Male mice were sacrificed at termination of matings, and female mice were sacrificed 12 days after the last cohabitation day. The reproductive status, total number, position and status of implantations, the number of early and late resorptions, and the number of live and dead fetuses were recorded.

No animals died during the study, and body weights of the exposed groups were similar to those of the control group. All males exposed to 1,3-butadiene were fertile during the 8-week exposure period. During the first week of mating (postexposure week), the total number of dead implants was significantly elevated for the group exposed only to 1,000 ppm ($p \leq 0.05$) compared with that of controls. Early resorptions accounted for most of the dead implants. In addition, the percentage of dead implants relative to the total implants was significantly elevated in groups exposed to 1,000 ppm ($p \leq 0.05$), and the percentage of females with more than one intrauterine death was significantly elevated in all exposed groups ($p \leq 0.05$) relative to controls. During the second postexposure week, the total number of dead implants was also significantly elevated at 200 and 1,000 ppm relative to controls. The percentage of dead implants and the percentage of females with more than one intrauterine death were elevated, but not significantly. For postexposure weeks 3, 5, 6, 7, and 8, the number of dead implantations, percentage of dead

implantations, and percentage of females with more than one intrauterine death in all exposed groups were similar to those of controls (i.e., not statistically significant). For postexposure week 4, the percentage of dead implants (5,000 ppm) and the percentage of females with more than one intrauterine death (200 and 5,000 ppm) were significantly reduced ($p \leq 0.05$) relative to the control value. However, the control values for these parameters were unusually high compared with control values at other postexposure weeks. Thus, the significantly reduced values for treated mice were probably not treatment related.

The results indicate that exposure to 1,3-butadiene may affect mature spermatozoa and spermatids assessed by preimplantation deaths for postexposure weeks 1 and 2. Interpretation of these results is complicated because the effects occurred in the 200 and 1,000 ppm groups but not in the 5,000 ppm group, which showed no indications of toxicity.

5.1.7. Anderson et al., 1993

The ability of 1,3-butadiene to induce dominant lethal mutations in male mice following acute and subchronic inhalation exposure was assessed by evaluating the number of dead implants in females mated to exposed males. For acute exposures, male CD-1 mice were exposed to 0, 1,250, and 6,250 ppm 1,3-butadiene for 6 h; 5 days later, each male was mated to two females. Males used for subchronic exposures were treated with 0, 12.5, or 1,250 ppm, 6 h/day, 5 days/week for 10 weeks. Following mating in both experiments, one female was killed on gestation day (gd) 17 and the other was allowed to litter for evaluation of long-term effects on the offspring. Results of long-term carcinogenic effects on the live offspring are not yet available. The female killed on gd 17 was examined for number of live fetuses, number and type of malformations in the fetuses, and number of postimplantation deaths. The only effect seen in the acute study was a decrease ($p \leq 0.05$) in the number of implantations in females mated to males exposed to 1,250 ppm. In the subchronic study, females mated to males exposed to 12.5 ppm had an increase in the number of late postimplantation deaths ($p \leq 0.01$; both fetal and placental tissue were present); females mated to males exposed to 1,250 ppm had a decrease in mean implantations per dam ($p \leq 0.01$) and an increase in both early ($p \leq 0.001$; resorption) and late postimplantation deaths ($p \leq 0.001$). 1,3-Butadiene appears to affect the male germ cell line, resulting in late postimplantation death of the fetuses. It is unknown whether the mutations/alterations of the germ cells resulting in a reduction in live fetuses are due to an effect on reproductive ability or a teratogenic effect resulting in death.

5.1.8. Adler et al., 1994

To assess the stage at which male germ cells are affected by 1,3-butadiene, male (102/E1 × C3H/E1)F_1 mice were exposed by inhalation to 0 or 1,300 ppm, 6 h/day for 5 consecutive days.

Four hours after the end of exposure, each male was mated at a ratio of 1:2 to untreated virgin females. Females judged bred by the presence of a vaginal plug were replaced with new females, and mating continued for 4 consecutive weeks. Females were killed on gd 14 to 16 and examined for numbers of live and dead implants. Exposure of male mice to 1,300 ppm resulted in an increase in dead implants during the first to the third weeks of mating; however, statistical significance ($p \leq 0.01$) was reached only in the second week. When expressed as a percentage of dominant lethals, a significant increase was seen in the second (12.4%, $p \leq 0.01$) and third (5.5%, $p \leq 0.05$) weeks. Because of the time course for dominant lethal mutations to manifest as dead implantations, 1,3-butadiene appears to induce dominant lethality in spermatozoa and late spermatids.

5.2. DEVELOPMENTAL EFFECTS

The developmental toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP, 1982) is the same as the Hazleton study discussed briefly in the 1985 EPA document (U.S. EPA, 1985). Hackett and coworkers also conducted two developmental toxicity studies, one using rats (Hackett et al., 1987a) and one using mice (Hackett et al., 1987b). The study using rats was conducted to confirm and extend the findings of the IISRP (1982) study in rats, and the mouse study was conducted for comparison of a rodent species more sensitive than the rat to the toxic effects of 1,3-butadiene.

5.2.1. IISRP, 1982

Female Sprague-Dawley CD rats were mated with male rats of the same strain (2f:1m) to produce 138 sperm-positive females. Groups of mated females (220 to 266 g) were exposed by inhalation to 1,3-butadiene at target concentrations of 0, 200, 1,000, or 8,000 ppm 6 h/day on gd 6 to 15 and killed on gd 20. Measured concentrations (mean ± SD) were 2.8 ± 1.2, 202 ± 14, 990 ± 24, and 7,647 ± 375 ppm for 0, 200, 1,000, and 8,000 ppm, respectively. The animals were exposed in stainless steel chambers. Twenty-four pregnant females were exposed to each concentration of 1,3-butadiene, 40 were exposed to filtered air (controls), and 26 were given 250 mg acetylsalicylic acid/kg body weight by gavage on gd 6 to 15 (positive controls). The purity of the 1,3-butadiene was not reported; the mean concentration of the dimer, 4-vinyl-1-cyclohexene, was 108.6 ± 53.59, well below the target of 300. The rats were weighed on gd 0, 3, 6, 9, 12, 15, 18, and 20. Various parameters of maternal and developmental toxicity were evaluated and analyzed using the litter as the statistical unit.

Maternal effects of 1,3-butadiene are summarized in Table 5-3. No animals died of exposure to 1,3-butadiene. One animal exposed to 1,000 ppm was killed because of morbidity unrelated to treatment. Clinical signs of toxicity were not observed in any group, and the

Table 5-3. Maternal toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	**200**	**1,000**	**8,000**
No. dams assigned	40	24	24	24
No. of deaths	0	0	1[a]	0
No. pregnant (%)	90	91.7	100	95.8
Whole body weight (g)				
Day 0	239	238	240	239
Day 20	362	357	355	347
Body weight gain[b] (g)				
Days 0-6	24	24	23	23
Days 6-9	13	9	1[c]	1[c]
Days 9-12	16	13	14	11[c]
Days 12-15	15	15	16	15
Days 15-20	54	58	61	60
Gravid uterine weight (g)	63.9	61.1	66.5	62.8
Extragestational weight[d] (g)	297.9	296.2	280.8	283.9
Extragestational weight gain[e] (g)	59	58	49[f]	45[f]
Significant clinical signs	None	None	None	None

[a]This animal was killed in moribund state on day 19; morbidity was not related to exposure to butadiene.
[b]Determined from differences in group mean body weights reported for specific days of gestation.
[c]$p<0.01$, compared with corresponding control; analysis of variance and *t* test.
[d]Body weight on gd 20 minus gravid uterine weight.
[e]Extragestational weight minus body weight on gd 0.
[f]$p<0.05$, compared with corresponding control; analysis of variance and *t* test.

Source: IISRP, 1982.

pregnancy rates were similar in all groups. Terminal body weights showed a dose-related decrease (no statistical analysis). Maternal body weight gain was markedly depressed in dams exposed to 1,000 and 8,000 ppm, especially during gd 6 to 9; a significant decrease was also noted during gd 9 to 12 in rats exposed to 8,000 ppm. During the later stages (gd 12 to 15 and 16 to 20), body weight gain was similar to controls. The gravid uterus and extragestational weights were similar to controls, but extragestational weight gain was significantly depressed by 17% ($p<0.05$) in dams exposed to 1,000 ppm and by 24% in dams exposed to 8,000 ppm ($p<0.05$). No effects were observed on other measures of maternal toxicity. Developmental effects of 1,3-butadiene are summarized in Tables 5-4 and 5-5. Fetal body weight and crown/rump length were significantly reduced at 8,000 ppm ($p<0.05$). The percentage of fetuses with major skeletal defects was significantly elevated at 1,000 and 8,000 ppm, and minor skeletal defects were significantly elevated only at the lowest concentration. The percentage of fetuses showing minor external/visceral defects, predominantly subcutaneous hematomas, was significantly elevated only at 1,000 ppm, but the percentage was similar in all three experimental groups. The incidence of bilateral lens opacity was elevated at all concentrations but was significantly elevated only at 8,000 ppm. The incidence of marked-to-severe wavy ribs and the total number of abnormal ossifications and irregular ossification of the ribs were elevated at 8,000 ppm. The incidence of thoracic bipartite centers was elevated in all exposed groups; a dose-response relationship was not observed. Other malformations and variations occurred at incidences similar to those of controls or were not significantly elevated compared with controls.

5.2.2. Hackett et al., 1987a

For the experiment with rats, 208 female Sprague-Dawley CD rats and 108 male Sprague-Dawley CD rats (all 7 to 8 weeks old) were used. The rats were mated by placing two females with one male rat overnight for 5 consecutive nights or until a sperm-positive vaginal smear was obtained; gd 0 was the day sperm were detected. Thirty sperm-positive female rats per group were exposed to 0, 40, 200, or 1,000 ppm 1,3-butadiene (99.84% purity; 197 ± 6 ppm mean headspace dimer concentration). The measured concentrations (mean ± SD) were 40.1 ± 0.62 (mean ± SD), 199.8 ± 2.61, and 1,005 ± 11.9 ppm, respectively. On gd 6 to 15, the females were exposed for 6 h/day in stainless-steel chambers having a total volume of 2.3 m^3 and a mixing volume of 1.7 m^3. The females were weighed 1 week before mating and on gd 0, 6, 11, 16, and 20 (the day of sacrifice). Various parameters of maternal and developmental toxicity were evaluated. The experimental design is summarized in the upper section of Table 5-6.

The effects of inhalation exposure to 1,3-butadiene on maternal endpoints in rats are summarized in Table 5-7. All females survived to the end of the study. No clinical signs of toxicity were observed. Final body weights were similar to those of controls; body weight gain,

Table 5-4. Developmental toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	**200**	**1,000**	**8,000**
No. pregnant (%)	90	91.7	100	95.8
No. litters with live fetuses	36	22	23	23
No. implantations/dam[a]	13.0	12.8	14.1	13.8
Preimplantation loss (%)	15.4	17.1	11.5	12.4
Postimplantation loss (%)	3.6	6.0	4.9	7.3
Total no. resorptions	17	17	16	23
Early resorptions	16	13	16	20
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	450/36	265/22	308/23	294/23
Fetal body weight[a] (g)	3.3	3.2	3.2	3.1[b]
Females[a]	3.2	3.1	3.1	3.0
Males[a]	3.4	3.3	3.3	3.2
Crown/rump length (mm)	37.8	37.2	37.2	35.9[c]
Sex ratio (% males)	49.8	54.7	51	50

[a]Mean values.
[b]$p<0.05$, Wilcoxon test.
[c]$p<0.01$, Wilcoxon test.

Source: IISRP, 1982.

Table 5-5. Malformations and variations in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	200	1,000	8,000
Total no. fetuses/no. litters examined	450/36	265/22	308/23	294/23
External/visceral defects				
Minor defects	76 (16.9)[a]	63 (23.8)	75 (24.4)[b]	75 (23.3)
Major defects	0	0	0	2 (0.7)
Unilateral lens opacity	19 (4.2)	11 (4.2)	8 (2.6)	13 (4.4)
Bilateral lens opacity	18 (4.0)	24 (9.1)	30 (9.7)	31 (9.5)[b]
Bilateral ureter dilation	13 (2.9)	8 (3.0)	3 (1.6)	19 (6.5)
Skeletal defects				
Minor defects	72 (23.2)	49 (26.9)[c]	45 (20.9)	43 (21.1)
Major defects	2 (0.6)	4 (2.2)	6 (2.8)[b]	12 (5.9)[d]
Any thoracic center (10-13) bipartite	4 (1.29)	11 (6.04)[d]	14 (6.51)[d]	8 (3.92)[d]
Wavy ribs (marked to severe)	2 (0.6)	4 (2.2)	3 (2.3)	7 (3.4)[b]
Wavy ribs (slight to moderate)	3 (1.6)	3 (1.7)	7 (3.3)	8 (3.9)
Variations (abnormal ossification)	267 (85.9)	164 (90.1)	185 (84.0)	199 (97.5)[c]
Skull (occipital)	79 (25.4)	58 (31.9)	69 (32.1)	71 (34.8)
Skull (interparietal)	82 (26.4)	66 (36.3)	79 (36.7)	75 (36.7)
Sternebrae no. 6	152 (48.9)	107 (58.8)	126 (58.6)	147 (72.1)
Ribs	0	1 (0.55)	2 (0.93)	6 (2.9)[b]
Metacarpals	207 (66.6)	140 (76.9)	141 (65.6)	172 (84.3)
Phalanges	141 (45.3)	114 (62.6)	139 (64.6)	123 (60.3)

[a]Numbers of fetuses affected; numbers in parentheses denote the percentage of affected fetuses/fetuses examined.
[b]$p<0.05$, Fisher's randomization test based on frequencies of affected litters.
[c]$p<0.05$, Wilcoxon's test.
[d]$p<0.01$, Fisher's randomization test based on frequencies of affected litters.

Source: IISRP, 1982.

Table 5-6. Design of the developmental toxicity studies on 1,3-butadiene

Species/strain/route of exposure	Exposure (ppm)	No. of animals/ group	Gestation days of exposure	Gestation day of sacrifice
Rat/ Sprague-Dawley CD/ Inhalation	0	30	6-15	20
	40	30	6-15	20
	200	30	6-15	20
	1,000	30	6-15	20
Mouse/ CD-1/Inhalation	0	32	6-15	18
	40	33	6-15	18
	200	31	6-15	18
	1,000	33	6-15	18

Source: Hackett et al., 1987a, b.

Table 5-7. Maternal toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	**40**	**200**	**1,000**
No. dams assigned	30	30	30	30
No. of deaths	0	0	0	0
No. pregnant (%)	28 (93)	24 (80)	26 (87)	28 (93)
Whole body weight (g)				
Day 0	242 ± 3.7[a]	239 ± 3.2	244 ± 3.0	242 ± 4.0
Day 20	362 ± 7.1	351 ± 5.9	369 ± 6.6	354 ± 6.1
Body weight gain (g)				
Days 0-6	21.4 ± 1.6	21.1 ± 1.6	22.9 ± 1.3	20.1 ± 1.5
Days 6-11	25.5 ± 1.3	23.6 ± 1.3	26.6 ± 1.5	17.5 ± 1.9[b]
Days 11-16	29.2 ± 1.4	30.9 ± 1.7	31.7 ± 1.9	31.2 ± 2.1
Days 16-20	44.5 ± 1.8	36.7 ± 2.5	43.6 ± 2.3	43.2 ± 2.9
Gravid uterine weight (g)	73.0 ± 2.9	69.5 ± 3.5	73.9 ± 2.8	71.2 ± 4.1
Extragestational weight[c] (g)	289 ± 5.7	282 ± 3.9	295 ± 5.8	283 ± 3.5
Extragestational weight gain[d] (g)	47.6 ± 2.8	42.7 ± 2.2	50.9 ± 3.0	39.9 ± 3.5[b]
Significant clinical signs	None reported	None reported	None reported	None reported

[a]Mean ± standard error.
[b]$p<0.05$, compared with corresponding control.
[c]Body weight on gd 20 minus gravid uterine weight.
[d]Extragestational weight minus body weight on gd 0.

Source: Hackett et al., 1987a.

however, was reduced by about 30% ($p<0.05$) in the 1,000 ppm group during the first 5 days of exposure (gd 6 to 11). From gd 11 to 20, body weight gain was not significantly different from that of controls. The gravid uterine weights and extragestational weights (whole body weight minus gravid uterine weight) were similar to those of controls, but extragestational weight gain was significantly lower (16%; $p<0.05$) in dams exposed to 1,000 ppm than in control dams.

The overall pregnancy rates were similar among all groups, ranging from 80% among dams exposed to 40 ppm to 93% among controls and dams exposed to 1,000 ppm (Table 5-7). Fetal measures, including the numbers of implantations/dam, resorptions/litter, dead fetuses/litter, fetal body weights, sex ratios, malformations, and variations, were not affected by exposure to 1,3-butadiene (Tables 5-8 and 5-9). Overall, no developmental toxicity was observed in rats exposed to 40 to 1,000 ppm during gd 6 to 15; a slight maternal toxicity, manifested as reduced extragestational weight gain, was observed at the 1,000 ppm level.

5.2.3. Hackett et al., 1987b

Because 1,3-butadiene is more toxic in mice than in rats, a study was also conducted in CD-1 mice using a protocol similar to that used for the rats. Groups of 31 to 33 sperm-positive females were exposed to 0 (filtered air), 40, 200, or 1,000 ppm 1,3-butadiene (99.88% purity; 338 ± 72 ppm mean headspace dimer concentration), 6 h/day on gd 6 to 15 (Table 5-6, bottom section). Measured concentrations were 39.9 ± 0.06, 199.8 ± 3.0, and 1,000 ± 13.1 ppm (mean ± SD). The dams were weighed on gd 0, 6, 11, 16, and 18 (day of sacrifice).

The effects of 1,3-butadiene on maternal toxicity in CD-1 mice are summarized in Table 5-10. Three animals exposed to 1,000 ppm showed signs of dehydration: two died on gd 15, and early parturition occurred in the third. No other clinical signs of toxicity were observed. Exposure-related decreases in whole body weights on gd 18, body weight gain during gd 11 to 16, gravid uterine weight, extragestational weight, and extragestational weight gain were significantly reduced in the 1,000 ppm exposure group compared with controls. Whole body weight gain during gd 11 to 16 and extragestational weight gain was also reduced in the 200 ppm exposure group. None of these parameters were significantly affected in dams exposed to 40 ppm. The pregnancy rates in mice were uniformly low in all groups and unaffected by exposure to 1,3-butadiene. The effects of 1,3-butadiene on various parameters of developmental toxicity in CD-1 mice are summarized in Tables 5-11 and 5-12. More resorptions per litter were observed among control dams than among exposed dams. Fetal body weights were reduced in all exposed groups compared with controls, and the reduction showed a significant exposure-related trend. The overall fetal body weights (males and females combined) were reduced by 4.5% at 40 ppm (not significant), 15.7% at 200 ppm ($p\leq0.05$), and 22.4% at 1,000 ppm ($p\leq0.05$). Significant differences from controls were seen at all treatment concentrations for fetal males and

Table 5-8. Developmental toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. pregnant (%)	28 (93)	24 (80)	26 (87)	28 (93)
No. litters with live fetuses	28	24	26	27[a]
No. implantations/dam	14.4 ± 0.55[b]	14.0 ± 0.71	15.3 ± 0.45	14.8 ± 0.63
No. resorptions/litter	0.46 ± 0.17	0.58 ± 0.17	0.96 ± 0.26	0.67 ± 0.14
Early resorptions/litter	0.39 ± 0.15	0.54 ± 0.16	0.88 ± 0.25	0.63 ± 0.14
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	389/28	321/24	372/26	382/27
Fetal body weight (g)	3.49 ± 0.04	3.44 ± 0.05	3.40 ± 0.05	3.50 ± 0.06
Females	3.40 ± 0.05	3.36 ± 0.05	3.29 ± 0.06	3.38 ± 0.06
Males	3.59 ± 0.05	3.52 ± 0.05	3.51 ± 0.06	3.59 ± 0.06
Sex ratio (% males)	50.2 ± 2.281	52.5 ± 2.95	50.5 ± 2.77	52.5 ± 2.58

[a]One rat had only one implant; this animal was excluded from statistical evaluations.
[b]Mean ± standard error.

Source: Hackett et al., 1987a.

Table 5-9. Malformations and variations in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. fetuses/no. litters examined	389/28	321/24	372/26	382/27
No. fetal heads examined	196	161	185	191
Malformations[a]				
Generalized edema	1/1	3/1	1/1	3/1
Hydrocephalus	--[b]	3/3	--	--
Meningoencephalocele	--	--	--	2/1
Missing rib	--	--	2/2	--
Variations				
Low ear set	--	--	2/1	--
Hydroureter	36/17	35/15	39/14	31/12
Misaligned sternebrae	--	--	1/1	1/1
Extra rib	--	1/1	4/2	--
Reduced ossification				
Skull	27/13	22/13	18/10	29/11
Sternebrae no. 1-4	60/15	48/13	95/21	66/15
Ribs	1/1	2/2	5/3	2/2
Thoracic vertebrae (centra)	109/23	97/21	75/21	81/25
Pelvis	9/7	--	6/5	5/5
Phalanges	1/1	6/1	2/1	--

[a]Expressed as number of fetuses/number of litters; includes only those findings occurring in more than one fetus or at more than one concentration.
[b]--, no malformations observed.

Source: Hackett et al., 1987a.

Table 5-10. Maternal toxicity in pregnant CD-1 mice exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	**40**	**200**	**1,000**
No. dams assigned	32	33	31	33
No. of deaths	0	0	0	3
No. pregnant (%)	18 (56)	19 (57)	21 (68)	22 (67)
Whole body weight (g)				
Day 0	28.4 ± 0.25[a]	28.3 ± 0.32	28.2 ± 0.32	28.4 ± 0.32
Day 18	54.9 ± 1.21[b]	55.4 ± 1.09	52.5 ± 1.01	50.8 ± 0.86[c]
Body weight gain (g)				
Days 0-6	2.7 ± 0.3	3.0 ± 0.3	2.5 ± 0.2	2.3 ± 0.2
Days 6-11	5.5 ± 0.4	5.8 ± 0.3	5.6 ± 0.3	4.8 ± 0.3
Days 11-16	13.3 ± 0.6[b]	12.7 ± 0.4	11.4 ± 0.5[c]	10.6 ± 0.4[c]
Days 16-18	5.5 ± 0.3[b]	5.7 ± 0.3	4.7 ± 0.4	4.8 ± 0.3
Gravid uterine weight (g)	19.3 ± 1.00[b]	20.3 ± 0.80	18.0 ± 0.87	16.8 ± 0.67[c]
Extragestational weight[d] (g)	35.5 ± 0.48[b]	35.1 ± 0.44	34.5 ± 0.46	34.1 ± 0.36[c]
Extragestational weight gain[e] (g)	7.60 ± 0.48[b]	6.99 ± 0.38	6.20 ± 0.38[c]	5.91 ± 0.28[c]
Significant clinical signs	None	None	None	Dehydration

[a]Mean ± standard error.
[b]$p \leq 0.05$, significant linear trend.
[c]$p \leq 0.05$, pairwise comparison with corresponding control parameter.
[d]Body weight on gd 18 minus gravid uterine weight.
[e]Extragestational weight minus body weight on gd 0.

Source: Hackett et al., 1987b.

Table 5-11. Developmental toxicity in CD-1 mice exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. pregnant (%)	18 (56)	19 (57)	21 (68)	22 (67)
No. litters with live fetuses	18	19	21	20
No. implantations/dam	12.7 ± 0.52	13.3 ± 0.44	13.0 ± 0.64	13.1 ± 0.43
No. resorptions/litter	1.06 ± 0.22	0.84 ± 0.22	0.67 ± 0.20	0.90 ± 0.19
Early resorptions	1.00 ± 0.23	0.58 ± 0.21	0.43 ± 0.13[a]	0.75 ± 0.16
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	11.7 ± 0.66	12.5 ± 0.52	12.3 ± 0.62	12.2 ± 0.51
Fetal body weight (g)	1.34 ± 0.03[b]	1.28 ± 0.01	1.13 ± 0.02[a]	1.04 ± 0.03[a]
Females	1.30 ± 0.03[b]	1.25 ± 0.01	1.10 ± 0.02[a]	1.06 ± 0.02[a]
Males	1.38 ± 0.03[b]	1.31 ± 0.02[a]	1.13 ± 0.02[a]	1.06 ± 0.02[a]
Placental weight (mg)	86.8 ± 2.99[b]	85.4 ± 2.29	78.6 ± 3.24[a]	72.6 ± 1.88[a]
Females	83.1 ± 3.03[b]	80.9 ± 2.46	74.7 ± 3.52[a]	70.1 ± 2.33[a]
Males	89.3 ± 3.03[b]	89.5 ± 2.27	80.1 ± 2.35[a]	74.5 ± 1.81[a]
Sex ratio (% males)	51.6 ± 3.91	49.8 ± 3.06	51.5 ± 3.68	51.8 ± 3.29

[a]$p \leq 0.05$, pairwise comparison with corresponding control.
[b]$p \leq 0.05$, significant linear trend.

Source: Hackett et al., 1987b.

Table 5-12. Malformations and variations in CD-1 mice exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. fetuses/no. litters examined	211/18	237/19	259/21	244/20
No. fetal heads examined	105	120	130	120
Malformations[a]				
Exencephalus	1/1	--[b]	--	2/2
Open eye	1/1	--	--	1/1
Limb flexure	2/1	--	--	--
Fused sternebrae	—	--	—	2/2
Fused ribs	--	2/2	--	--
Variations				
Pale	2/2	--	--	--
Hydroureter	2/2	6/3	--	--
Abnormal sternebrae[c,d]	0.6 ± 0.9	0.4 ± 0.7	0.4 ± 0.8	0.8 ±1.3[e]
Misaligned sternebrae	10/6	3/3	9/8	10/8
Ossification site between sternebrae 5 and 6	--	1/1	1/1	3/3
Supernumerary ribs[c,d]	1.7 ± 2.3	1.6 ± 2.1	6.0 ± 3.6[e]	9.9 ± 3.0[e]
Supernumerary ribs (total number)	30/11	30/9	127/20	198/20
Normal length	6/5	5/1	29/9	68/10
Rudimentary	13/6	19/8	81/20	120/16
Ossification site at lumbar 1	11/5	6/4	17/10	10/7

Table 5-12. Malformations and variations in CD-1 mice exposed to 1,3-butadiene by inhalation (continued)

Parameter	Concentration (ppm)			
	0	40	200	1,000
Reduced ossification (all sites)[c]	1.7 ± 1.7	1.2 ± 1.5	2.7 ± 2.7	3.9 ± 2.6[e]
Skull	--	--	2/2	3/1
Sternebrae	31/13	20/9	57/16[f]	76/19[f]
Vertebrae (centra)	--	1/1	--	1/1
Phalanges	--	--	--	2/16

[a]Expressed as number of fetuses/number of litters; includes only those findings occurring in more than one fetus or at more than one concentration.
[b]--, no malformations or variations noted.
[c]Mean percentage per litter (mean ± SD).
[d]$p<0.05$, linear trend, orthogonal contrast test.
[e]$p<0.05$, Tukey's test.
[f]$p<0.05$, Fisher exact test (fetal incidence).

Source: Hackett et al., 1987b.

at the two higher concentrations for females. Placental weights showed an effect similar to that of fetal body weights (Table 5-11). Malformations occurred sporadically and at low frequencies in all exposure groups (Table 5-12). The frequency of supernumerary ribs was greatly elevated at 200 and 1,000 ppm; 6% of the fetuses/litter were affected at 200 ppm ($p<0.05$) and 9.9% at 1,000 ppm ($p<0.05$) compared with 1.7% in controls and 1.6% in the 40 ppm exposure group (not significant). There also was a marked increase in the total number of fetuses with supernumerary ribs at the 200 and 1,000 ppm exposure levels. The frequency of reduced ossification of the sternebrae was elevated at 200 ($p<0.05$) and 1,000 ppm ($p<0.001$) (Fisher exact test); the litter incidence was elevated but not significantly. The percentages of reduced ossifications at all sites and the percentages of abnormal sternebrae (misaligned, scrambled, or cleft) per litter were also significantly elevated at 1,000 ppm ($p<0.05$). The percentages of supernumerary ribs and abnormal sternebrae also showed significant linear trends.

These studies showed that inhalation exposure to 1,3-butadiene causes maternal toxicity, manifested as reduced body weight gain, in the mouse at 200 and 1,000 ppm; therefore, the NOAEL for maternal toxicity is 40 ppm. 1,3-Butadiene also caused developmental effects, manifested by reduced fetal body weight and increased frequency of skeletal variations at 200 and 1,000 ppm. In addition, inhalation exposure to 1,3-butadiene during gestation caused a significant reduction in body weight of male fetuses at 40 ppm. Therefore, a NOAEL for developmental toxicity in CD-1 mice could not be obtained. Although 1,3-butadiene did not induce gross malformations in the mouse fetus, the dose-related increases in supernumerary ribs and reduced ossifications, particularly of the sternebrae, may indicate delayed or altered development and should be cause for concern.

5.2.4. Anderson et al., 1993

The acute and subchronic effects of inhalation exposure to 1,3-butadiene in male mice on fetal abnormalities were examined. For acute exposures, male CD-1 mice were exposed to 0, 1,250, and 6,250 ppm 1,3-butadiene for 6 h; 5 days later each male was mated to two females. Males used for subchronic exposures were treated with 0, 12.5, or 1,250 ppm 6 h/day, 5 days/week for 10 weeks. Following mating in both experiments, one female was killed on gd 17 and the other was allowed to litter. The female killed on gd 17 was examined for number of live fetuses, number and type of malformations in the fetuses, and number of postimplantation deaths. No treatment-related abnormalities were observed in offspring of males treated on the acute exposure protocol; one fetus from one control litter had a gastroschisis (fissure of abdominal cavity), and one fetus from each of the two low-dose litters was a runt (body weight $\leq$67% of mean litter weight). Following subchronic exposure of males to 1,3-butadiene, 7 of 306 fetuses sired by males exposed to 12.5 ppm ($p\leq0.05$) and 3 of 406 fetuses sired by males exposed to

1,250 ppm were affected compared with 0 of 278 fetuses sired by control males. Abnormalities in low-dose fetuses included four exencephalies, two runts (≤70% of mean litter weight), and one fetus with blood in the amniotic sac. At the high-dose level, one hydrocephaly and two runts (≤75% of mean litter weight) were observed. The authors calculated statistical significance on a fetal incidence basis rather than on a litter incidence basis. Because litter incidence rates were not included in the data, it is not possible to discern whether the affected fetuses were only from one or two litters or whether a high percentage of litters sired by exposed males were affected. Therefore, this study is inadequate to assess the developmental toxicity of 1,3-butadiene following exposure of males prior to mating.

5.3. STRUCTURE-ACTIVITY RELATIONSHIPS

Data on structure-activity relationship are summarized in Table 5-13.

5.3.1. NTP, 1986

The 1,3-butadiene dimer, 4-vinylcyclohexene, and its diepoxide, 4-vinyl-1-cyclohexene diepoxide, have been tested in long-term toxicological and carcinogenicity studies in rats and mice. The NTP study (1986) on 4-vinylcyclohexene used male and female F344 rats and male and female B6C3F$_1$ mice dosed by gavage with 0, 200, or 400 mg/kg 4-vinylcyclohexene in corn oil 5 days/week for 105 weeks. No nonneoplastic lesions attributed to exposure to 4-vinylcyclohexene were observed in the reproductive organs of male or female mice or rats, and hence data are not presented in Table 5-13. The incidences of granulosa cell neoplasms, mixed benign tumors, granulosa cell hyperplasia, and tubular cell hyperplasia were increased in female mice. Tubular cell hyperplasia is a proliferative lesion originating in the germinal epithelium; the hyperplastic cells invade the ovarian stroma forming tubular structures. The granulosa cell hyperplasia is a morphological continuum with granulosa cell neoplasms and tubular hyperplasia with mixed benign tumors and therefore should not be included with nonneoplastic lesions. The authors noted that female mice treated with 1,200 mg/kg 4-vinylcyclohexene for 5 days/week for 13 weeks had reduced numbers of primary and mature Graafian follicles whether they survived until termination (5/10) or died before termination (5/10).

In another NTP study (1989), male and female F344 rats and B6C3F$_1$ mice were treated topically with 4-vinyl-1-cyclohexene diepoxide 5 days/week for 13 weeks and 2 years. No nonneoplastic lesions occurred in reproductive organs of rats. Female mice treated for 13 weeks, however, showed evidence of diffuse ovarian atrophy in 10/10 animals that received 10 mg/mouse (highest dose) and in 4/10 receiving 5 mg/mouse. Uterine atrophy was observed in 2/10 animals that received 10 mg/kg. In the 2-year study, ovarian atrophy occurred in almost

Table 5-13. Reproductive and developmental toxicity of chemicals structurally similar to 1,3-butadiene

Chemical	Species	Dose	Effects	LOAEL	Reference
4-vinyl-1-cyclohexene	Male and female $B6C3F_1$ mice	2.5, 5, 10 mg/kg topically 5 days/week for 13 or 105 weeks	Females 13 weeks: ≥5 mg/kg: ovarian atrophy; 10 mg/kg: uterine atrophy 105 weeks: ≥2.5 mg/kg: ovarian atrophy Males 105 weeks: ≥5 mg/kg: inflammation of epididymis	5 mg/kg for 13 weeks 2.5 mg/kg for 105 weeks 5 mg/kg for 105 weeks	NTP 1989
Butadiene monoepoxide	Female $B6C3F_1$ mice	0.005, 0.02, 0.09, 0.36, 1.43 mmol/kg intraperitoneally once daily for 30 days	1.43 mmol/kg: reduced ovarian and uterine weights; decreased follicular counts	1.43 mmol/kg	Doerr et al., 1995, 1996
	Female Sprague-Dawley rats		none		Doerr et al., 1996
Butadiene diepoxide	Female $B6C3F_1$ mice	0.002, 0.009, 0.036, 0.14, 0.29 mmol/kg intraperitoneally once daily for 30 days	≥0.14 mmol/kg: decreased ovarian and uterine weights; decreased follicular counts	0.14 mmol/kg	Doerr et al., 1995, 1996
	Female Sprague-Dawley rats		≥0.14 mmol/kg: decreased ovarian weight 0.29 mmol/kg: decreased uterine weights	0.14 mmol/kg	Doerr et al., 1996
Isoprene	Male $B6C3F_1$ mice	70, 220, 700, 2,200, 7,000 ppm inhalation 6 h/day, 5 days/week, 13 or 26 weeks	13 and 26 weeks: 7,000 ppm: testicular atrophy	7,000 ppm	Melnick et al., 1994
	Male F344 rats		13 weeks: none 26 weeks: 7,000 ppm: hyperplasia of interstitial cells	7,000 ppm for 26 weeks	Melnick et al., 1994

all groups treated with 2.5, 5, and 10 mg/mouse (43/49, 42/49, and 47/50, respectively, compared with 12/50 for controls). Ovarian atrophy such as that in animals exposed to 1,3-butadiene was characterized by a complete absence of follicles and corpora lutea. Tubular hyperplasia occurred at a high incidence in all dose groups (35/49, 38/49, and 34/50, respectively, compared with 5/50 for controls). In male mice, subacute inflammation of the epididymis occurred in 0/50, 6/50, and 13/49, respectively, compared with 0/50 for controls.

5.3.2. Melnick et al., 1994

Differences in susceptibility between rats and mice were seen in inhalation studies with isoprene, the 2-methyl analogue of 1,3-butadiene. Male F344 rats and $B6C3F_1$ mice were exposed to 0, 70, 220, 700, 2,200, and 7,000 ppm isoprene 6 h/day, 5 days/week for either 13 weeks or 26 weeks followed by a 26-week recovery period. After 13 weeks of exposure, no effects were observed in rats at any concentration, but testicular atrophy occurred in mice at 7,000 ppm. Following 26 weeks of exposure, all treated rats in the 7,000 ppm group had hyperplasia of the interstitial cells of the testis ($p \leq 0.01$; 10/10 vs. 1/10 controls); however, following the 26-week recovery, there was only a marginal increase (not significant) in benign testicular tumors: 9/30 compared with 3/30 for controls. Mice also had an increase in the incidence of testicular atrophy following 26 weeks of exposure to 7,000 ppm ($p \leq 0.05$; 5/10 vs. 0/10 controls). After 26 weeks of recovery, mice had a slight increase (not significant) in testicular atrophy at 7,000 ppm (3/29 compared with 0/29 for controls).

5.3.3. Doerr et al., 1996

This study tested the ovarian toxicity of the mono- and diepoxide metabolites of 1,3-butadiene in mice and rats. Butadiene monoepoxide (0.005, 0.02, 0.09, 0.36, or 1.43 mmol/kg), butadiene diepoxide (0.002, 0.009, 0.036, 0.14, or 0.29 mmol/kg), or vehicle (sesame oil) was administered intraperitoneally once daily to female $B6C3F_1$ mice and Sprague-Dawley rats for 30 days. Following day 30, animals were sacrificed by CO_2 inhalation, the ovaries and uteri were weighed, and the ovaries processed for histologic examination of preantral follicles. At the high dose, the monoepoxide resulted in reduced ovarian and uterine weights ($p \leq 0.05$) and decreased follicular counts in mice; rats, however, were unaffected. The diepoxide resulted in decreased ovarian weights ($p \leq 0.05$) in mice and rats at ≥ 0.14 mmol/kg and decreased uterine weights ($p \leq 0.05$) in mice at ≥ 0.14 mmol/kg and in rats at 0.29 mmol/kg. Because organ weights were given in a histogram, the absolute differences were not available; all significant reductions appeared to be approximately $\leq 50\%$ of the control values. The ED_{50} value was defined as the effective dose that reduces the follicular number to 50% of control. In mice, ED_{50} values for the monoepoxide were 0.29 and 0.40 mmol/kg and for the diepoxide were 0.1 and 0.14 mmol/kg for

small and growing follicles, respectively. However, in rats, only 32% of the follicular population was depleted at the highest diepoxide concentration. Therefore, mice were more sensitive than rats to the ovotoxic effects of the mono- and diepoxides of 1,3-butadiene, and the diepoxide was the more potent ovotoxicant in both species.

Doerr et al. (1995) also studied the ovarian toxicity of 4-vinylcyclohexene and several related olefins, including butadiene mono- and diepoxide. Mice were administered 1.43 mmol/kg of the monoepoxide or 0.14 mmol/kg of the diepoxide once daily for 30 days. Following day 30, the mice were killed and the ovaries removed and sectioned for histologic examination. Mean follicle counts in mice treated with the monoepoxide were depleted 98% and 71% for small and growing follicles, respectively, compared with controls. In mice treated with the diepoxide, follicle counts were depleted 85% and 63% for small and growing follicles, respectively, compared with controls. Structural analogs of vinylcyclohexene that contain only a single unsaturated site (vinylcyclohexane, ethylcyclohexene, cyclohexene) and their monoepoxide metabolites were not ovotoxic to mice. On the other hand, butadiene monoepoxide, butadiene diepoxide, and isoprene were ovotoxic. The study showed a relationship between chemical reactivity, as assessed by nicotinamide alkylation, and ovotoxicity with vinyldiepoxide and butadiene diepoxide that was 3.5 to 10 times more reactive than their monoepoxide precursors and other structurally related monoepoxides. It can be concluded that those compounds that are metabolized to a diepoxide or are a diepoxide are ovotoxic.

5.4. SUMMARY AND CONCLUSIONS

Evidence has been presented showing that 1,3-butadiene induces reproductive and developmental effects in rodents. Although the studies conducted by Carpenter et al. (1944) examined reproductive toxicity in four different species (rat, guinea pig, rabbit, and dog), the experimental protocol and the results obtained are inadequate for evaluating reproductive toxicity. The three long-term toxicity studies conducted in Sprague-Dawley CD rats (Owen et al., 1987; Owen and Glaister, 1990) and B6C3F$_1$ mice (NTP, 1984, 1993) suggest that mice are much more sensitive than rats to the reproductive effects of 1,3-butadiene. Reproductive toxicity was not observed in either male or female rats exposed intermittently to 1,3-butadiene at concentrations up to 8,000 ppm for 2 years. However, ovarian atrophy was observed in female mice exposed to 6.25 to 625 ppm 1,3-butadiene.

Ovarian atrophy occurred in 39% of 49 mice at 6.25 ppm (the lowest concentration tested) only after exposure for 2 years, a time at which this condition is expected to appear in aged animals due to normal senescence mechanisms; however, it occurred in a significantly greater number of mice exposed to 1,3-butadiene than in control animals. Furthermore, ovarian atrophy was observed as early as 9 months after exposure to 200 and 625 ppm and 15 months

after exposure to 62.5 ppm. Therefore, the dose-response relationship observed for ovarian atrophy and the significant increase at the lowest dose relative to that seen in control animals of a similar age is evidence for a causal relationship between ovarian atrophy and exposure to 1,3-butadiene at 6.25 ppm.

Similar ovarian lesions have been observed in mice after exposure to the 1,3-butadiene dimer, 4-vinylcyclohexene, administered by gavage for 13 weeks at 1,200 mg/kg for 5 days/week (NTP, 1986) or its diepoxide, 4-vinyl-1-cyclohexene diepoxide, administered by topical application for 13 weeks or 2 years (NTP, 1989). Rats administered 4-vinylcyclohexene or 4-vinyl-1-cyclohexene diepoxide did not develop ovarian lesions, thus showing a species-specific response similar to that after exposure to 1,3-butadiene.

Ovarian lesions induced by 1,3-butadiene, 4-vinylcyclohexene, or 4-vinyl-1-cyclohexene diepoxide are characterized by the absence of oocytes, follicles, and corpora lutea. The functional integrity of the reproductive system in animals exposed to 1,3-butadiene has not been tested, but the severity of the ovarian lesion is indicative of reproductive dysfunction. Furthermore, Maronpot (1987) compared the ovarian toxicity and carcinogenicity of eight chemicals tested by NTP and concluded that the occurrence of ovarian lesions in a 90-day study may also indicate that ovarian neoplasia would be induced upon continued treatment.

Uterine atrophy is probably due to the indirect action of 1,3-butadiene metabolites and the consequent interruption of ovarian sex steroid stimulation of the uterus. Oocyte toxicity and destruction of the follicular and subsequent luteal components of the ovary result in reduced steroidogenesis by the ovary. It is well known that ovarian estrogens and progestins have a uterotropic function in laboratory rodents and humans.

Testicular atrophy, as reflected by reduced testis weight following 1,3-butadiene exposure for 9 and 15 months in male mice (NTP, 1993) indicates gonadal sensitivity in the male as well as in the female. However, the ovary is more sensitive than the testis because ovarian atrophy results at very low concentrations (6.25 ppm) of 1,3-butadiene compared with that seen in males after 2 years of exposure. The sperm-head morphology study showed that male mice are affected at concentrations $\geq$1,000 ppm (Hackett et al., 1988a), and the dominant lethal test showed that male mice may be affected at 200 and 1,000 ppm (Hackett et al., 1988b), again indicating that higher exposure concentrations are necessary to induce toxic effects in male mice than in female mice. As observed for other effects of 1,3-butadiene, the reproductive organs of male rats are more resistant than those of female mice exposed to 1,3-butadiene. This resistance in males may be attributed, in part, to the blood-testis barrier. No homologous anatomical barrier has been demonstrated in the Graafian follicle (Crisp, 1992). No adverse reproductive effects have been observed in male rats at concentrations up to 8,000 ppm (Owen et al., 1987; Owen and Glaister, 1990).

Several studies indicate that 1,3-butadiene affects spermatozoa and spermatids as determined by postimplantation deaths during the first 3 weeks after exposure. The data from Hackett et al. (1988b) is equivocal because of a lack of the dose-response relationship, but the studies by Anderson et al. (1993) and Adler et al. (1994) confirm the hypothesis. Further evidence that 1,3-butadiene is a germ cell mutagen was presented in a comparison of the latter two studies (Adler and Anderson, 1994). In the first experiment, the percentage of dominant lethality observed following 10 weeks of exposure of males to 1,250 ppm was 28.1%. During the acute exposure experiment, the sum of dominant lethality over the 3 weeks of mating was 23.1%. The results are in close agreement despite differences in protocols such as exposure regimen, strains of mice, and mating scheme. It appears that 1,3-butadiene affects spermatozoa and spermatids because the effects observed after 10 weeks are representative of the last 3 weeks of treatment and increasing the length of exposure did not add to the response.

The mechanism by which 1,3-butadiene induces ovarian lesions is not known, but it is unlikely that 1,3-butadiene is a direct-acting reproductive toxicant. Direct-acting compounds act as hormonal agonists or antagonists or chemically reactive compounds (such as alkylating agents), which directly interfere with hormone-receptor interactions or interact with macromolecules (Maronpot, 1987; Mattison et al., 1990). More likely, 1,3-butadiene is an indirect-acting reproductive toxicant. Indirect-acting toxicants require metabolic activation to exert their toxic effects, which then may proceed via mechanisms similar to those of direct-acting compounds, or they may interfere with endocrine homeostasis (Mattison et al., 1990).

Developmental effects observed after exposure to 1,3-butadiene consisted primarily of reduced fetal body weight and minor skeletal defects such as abnormal ossifications, abnormal sternebrae, and supernumerary ribs. No gross malformations were produced. The pattern for developmental effects induced by 1,3-butadiene was similar to that of reproductive effects, with mice showing greater sensitivity than rats. This difference was also seen for maternal toxicity as manifested by decreased weight gain (whole body and extragestational) in both rats and mice. The NOAEL for maternal effects is 200 ppm in rats (IISRP, 1982; Hackett et al., 1987a) and 40 ppm for mice (Hackett et al., 1987b). While the IISRP (1982) study showed increased frequencies for bipartite thoracic centers and minor skeletal defects combined at 200 ppm in rats, the response is not clearly dose-related. Several developmental effects occurred at significantly increased frequencies at 8,000 ppm and showed a dose-response relationship: major skeletal defects combined, wavy ribs, and abnormal ossification of the ribs (IISRP, 1982). The results from the IISRP (1982) study were not confirmed in the more recent study by Hackett et al. (1987a), which showed no developmental toxicity in the same rat strain similarly exposed to 1,3-butadiene at concentrations up to 1,000 ppm. Therefore, the NOAEL for developmental effects in rats is 1,000 ppm (IISRP, 1982; Hackett et al., 1987a). These independent observations

strengthen the evidence that a decreased maternal weight gain during early development might contribute to subtle adverse effects (1) undetected by insensitive indices in the studies or (2) at a later point in time in the F_1 or subsequent generations. An NOAEL for developmental effects could not be defined for the mouse, because male fetal body weight was decreased at 40 ppm in the Hackett et al. (1987b) study and postimplantation loss was observed at 12.5 ppm in the Anderson et al. (1993) study, the lowest concentrations tested.

Reproductive and developmental toxicity studies show species specificity for exposure to 1,3-butadiene in rats and mice. Like other toxicological effects induced by 1,3-butadiene, mice are more sensitive than rats to the induction of reproductive and developmental effects. Pharmacokinetic studies show that uptake of 1,3-butadiene is about four times greater in mice than in rats at concentrations up to 1,000 ppm (Dahl et al., 1990) and about two times greater at 8,000 ppm (Dahl et al., 1991). These data indicate that the availability of 1,3-butadiene is greater in mice than in rats at comparable exposure concentrations. Nose-only exposure of mice and rats to 1,3-[^{14}C]-butadiene resulted in greater or similar concentrations of radioactivity (expressed as nM/g of tissue) in tissues of rats than those of mice under conditions in which the rats were exposed to a 10-fold higher concentration of 1,3-butadiene (Bond et al., 1987). If tissue uptake was expressed as 1,3-butadiene equivalents/µM inhaled 1,3-butadiene, however, radioactivity levels were 15 to 100 times higher in mice. Mammary tissue, which had 4.6-fold higher concentration in rats than in mice, was the only tissue analyzed that was relevant to evaluating reproductive effects of 1,3-butadiene. Because male animals were used, subcutaneous fat, which had similar levels of radioactivity as mammary tissue, probably contaminated the samples. The ovary, uterus, and testis, which are targets for 1,3-butadiene, were not analyzed by Bond et al. (1987). In a recent in vitro study, Sharer et al. (1992) showed that microsomes from the testes of rats and mice are ineffective in forming butadiene monoxide, but the cytosol fraction was very effective in forming glutathione conjugates. Therefore, it is unlikely that toxic effects on the testes are due to metabolites formed within the testes but rather are due to metabolites formed elsewhere, indicating that 1,3-butadiene is an indirect-acting reproductive toxicant in males.

Species-specific differences have also have been observed for the formation of metabolites. The monoxide hydrolase (detoxification) pathway is favored in rat microsomes, whereas the monooxygenase pathway is favored in mouse microsomes. Although these data do not fully explain the species differences, they show that the basis of the difference may be related to the greater availability of 1,3-butadiene in mice, the greater production of toxic intermediates, and a lower capacity for detoxification of these intermediates.

No data are available regarding the reproductive or developmental effects of the metabolites of 1,3-butadiene, 1,2-epoxybutene, and diepoxybutane. Mice are more sensitive than rats to the ovotoxic effects of the mono- and diepoxides of 1,3-butadiene, and the diepoxide is the

more potent ovotoxicant in both species. Data regarding the 1,3-butadiene dimer 4-vinylcyclohexene and its diepoxide provide evidence that 4-vinylcyclohexene induces ovarian and uterine atrophy after treatment by gavage for 13 weeks with 10 mg/kg 5 days/week and that 4-vinyl-1-cyclohexene diepoxide (2.5 to 10 mg/mouse) induces ovarian atrophy and tubular hyperplasia in mice after topical treatment for 2 years. Subacute inflammation of the epididymis is seen in male mice receiving 4-vinyl-1-cyclohexene (5 or 10 mg/mouse) topically for 2 years. Ovarian neoplasms are induced in mice by 4-vinylcyclohexene and its diepoxide. However, neither 4-vinylcyclohexene nor its diepoxide induce either neoplastic or nonneoplastic lesions in the ovaries of rats.

In conclusion, the animal data show that there is a potential reproductive hazard to humans upon exposure to 1,3-butadiene, with women being more sensitive than men. The quantitative aspects of this assessment will require application of pharmacokinetic parameters because humans may be less sensitive than mice (Chapters 3, 8). The animal data also show that there is a potential for developmental effects in humans upon in utero exposure to 1,3-butadiene and that these effects may occur at concentrations below those causing maternal effects (Section 9.3).